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The goal of this DOD Breast Concept award was to identify and functionally characterize common genetic polymorphisms in the human UDP-glucuronosyltransferase gene, UGT1A9. We had previously determined that UGT1A9, a metabolic enzyme expressed predominantly in the human liver, catalyzes the glucuronidation and inactivation of the antiestrogen raloxifene (RAL). The pharmacokinetics of RAL is known to be subject to significant interindividual variation, possibly associated with variable clinical efficacy. We hypothesized that genetic variation in the human UGT1A9 gene contributed to the known variation in RAL pharmacokinetics in humans. The aims of this proposal were to 1) identify genetic polymorphisms within the coding regions of the human UGT1A9 gene, 2) functionally characterize recombinant UGT1A9 allozymes with regard to capacity to glucuronidate RAL and 3) express variant UGT1A9 cDNAs in MCF-7 cells and assess antiestrogenic response of cells to RAL.

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INTRODUCTION

The goal of this DOD Breast Concept award was to identify and functionally characterize common genetic polymorphisms in the human UDP-glucuronosyltransferase gene, UGT1A9. We had previously determined that UGT1A9, a metabolic enzyme expressed predominantly in the human liver, catalyzes the glucuronidation and inactivation of the antiestrogen raloxifene (RAL). The pharmacokinetics of RAL is known to be subject to significant interindividual variation, possibly associated with variable clinical efficacy. We hypothesized that genetic variation in the human UGT1A9 gene contributed to the known variation in RAL pharmacokinetics in humans. The aims of this proposal were to 1) identify genetic polymorphisms within the coding regions of the human UGT1A9 gene, 2) functionally characterize recombinant UGT1A9 allozymes with regard to capacity to glucuronidate RAL and 3) express variant UGT1A9 cDNAs in MCF-7 cells and assess antiestrogenic response of cells to RAL.

BODY

Aim 1. Identify common genetic polymorphisms in the human UGT1A9 gene. The UGT1A9 gene is part of a nested UGT1A gene family on human chromosome 2. The organization of this locus is such that alternative transcription initiation occurs at promoters of eight unique first exons, followed by splicing to common exons 2 through 5. Thus, eight unique UGT1A isoforms are expressed from this locus and those proteins differ in sequence at the amino terminal 530 amino acids by virtue of the unique exon 1 and they each share identical carboxy-terminal protein sequence that is encoded by the common exons 2 through 5. We and others have previously shown a lack of variation in gene sequence within the shared exons 2 through 5. Thus, genetic variation in UGT1A genes lies predominantly in the unique first exon. Therefore, we initially characterized common genetic variation in the UGT1A9-specific first exon.

Last year, we reported the identification of genetic polymorphisms within the 5'flanking region and 3' intron of UGT1A9. Table 1 describes those polymorphisms as well as their frequencies. Polymorphic loci were in genetic linkage such that different permutations of those polymorphisms defined eight apparent alleles. Of particular interest was the dT 9 or 10 variable length nucleotide repeat (VLNR) in the 5'flanking region of the gene. This position maps to the putative TATAA box of the UGT1A9 promoter.

Specific Aims 2 and 3. Functional Characterization of the UGT1A9 polymorphisms. Our original aims were to functionally characterize polymorphic UGT1A9 proteins (allozymes). However, none of the polymorphisms that we identified altered the encoded amino acid sequence of the protein. Therefore the experiments proposed would not be appropriate. Alternatively, we plan to evaluate the functional significance of the promoter polymorphism by comparing transcriptional activity of reporter constructs driven by the polymorphic promoter and by evaluating the correlation between level of

UGT1A9 transcript and genotype in cell culture systems. Progress on the latter aims has been hampered by a turnover in personnel and therefore an extension to this grant has been requested.

KEY RESEARCH ACCOMPLISHMENTS

- Identified five common genetic polymorphisms in the 5"flanking region and first intron of the human UGT1A9 gene.
- Determined the frequency and linkage of each of those polymorphisms in a population of 65 healthy Caucasian Americans.
- Currently evaluating the functional significance of the VLNR in the putative promoter.

Table 1. Genetic Variation in the Human UGT1A9 Gene

UGT1A9 Variable Loci	Nucleotide Polymorphism	Frequency
- 118 poly dT	T_9	0.56
	T_{10}	0.44
- 87	G	0.98
	A	0.02
I1 152	G	0.75
	A	0.25
I1 219	T	0.59
	A	0.41
I1 313	C	0.56
	A	0.44

-118 and -87 refer to nucleotide positions upstream of the "A" in the ATG start codon. I1 refers to intron 1 and the number following the "I1" designation refers to the nucleotide position downstream of the exon/intron junction.

REPORTABLE OUTCOMES

Jeffrey Zalatoris, Ph.D. and Rebecca Blanchard Raftogianis, Ph.D., UDP-glucuronosyltransferase-specific glucuronidation inactivates 4-hydroxytamoxifen and raloxifene. Oral Presentation at 2002 DOD Breast Cancer Era of Hope Meeting in Orlando, Fla.

CONCLUSIONS

We set out to characterize common genetic polymorphisms in the human UGT1A9 gene. Surprisingly, no polymorphisms affecting encoded amino acid sequence were identified. However, five common polymorphisms were identified in 5'flanking and intron regions of the gene. Of particular interest is the variable length nucleotide repeat in the putative promoter region of the gene. We are currently testing the hypothesis that this polymorphism is of functional consequence. Genetically determined variation in UGT1A9 activity may be an important factor in the clinical response of individuals to drugs that are metabolized via this pathway. This study has contributed toward our knowledge of common genetic variation in the human UGT1A9 gene.

REFERENCES

None

BIBLIOGRAPHY OF PUBLICATIONS

None

LIST OF PERSONNEL PAID FROM GRANT

Stephen Beaupariant, Postdoctoral Associate
Amanda Thistle, Scientific Technician